

Investigation between Alcoholic Extract and Essential Oil *Melissa officinalis* L. New in Growth Inhibition of *E. coli*

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Article information	Abstract
<p>Article history: Received: 16 Aug 2011 Accepted: 13 Dec 2011 Available online: 30 Dec 2012 ZJRMS 2013; 15(8): 42-45</p> <p>Keywords: <i>Melissa officinalis</i> Essential oil Anti-bacterial <i>E. coli</i></p> <p>*Corresponding author at: Department of Microbiology, Falavarjan Branch, Islamic Azad University, Esfahan. E-mail: nikbakhtzohre@yahoo.com</p>	<p>Background: Therapy of plant is not any side effects and drug resistant for inhibition of disease in world. In this study, antimicrobial effects of <i>Melissa officinalis</i> L. ethanol extract and essential oil on <i>E. coli</i> have been investigated.</p> <p>Materials and Methods: In this investigation, diameter of inhibitory zone of these materials was measured in disk agar diffusion method. Therefore, <i>Melissa officinalis</i> L. ethanolic extract (80%) and essential oil have been supplied. Then, antimicrobial activity these substances next for 24 hour for ethanol extraction 80% concentration 50 to 1000 mg/mL and essential oil concentration with 3% to 100% has been considered. Also, comparison of mean diameter of inhibitory zone between treatment and control groups of ANOVA has been used.</p> <p>Results: Results of these investigations were shown that extract ethanol <i>Melissa officinalis</i> L. was any inhibitory effect on <i>E. coli</i> growth 24 hour after of treatment. Also, diameter of inhibitory zone for 100% essential oil have been 33.2±0.13mm in <i>E. coli</i> that has been increased in comparison control groups significantly ($p=0.001$). This inhibitory effects was more than cefixime and ceftaxime ($p=0.00$, $p=0.01$).</p> <p>Conclusion: These found were shown that this plant prevents growth of <i>E. coli</i> In vitro condition. This essential oil could be suggested as antimicrobial agents for inhibition of bacterial diseases in human.</p> <p>Copyright © 2013 Zahedan University of Medical Sciences. All rights reserved.</p>

Introduction

Herbal remedies have been used for thousands of years. Early in human history, people practiced herbal medicine as a magical or religious healing art [1]. In this investigation was used *Melissa officinalis* (Lemon balm) and affects this plant on *Escherichia coli* that one inducement species infections at human were investigation [2].

Lemon balm (*Melissa officinalis* L.), member of Lamiaceae (formerly Labiatae) family, is one of the important medicinal plant species lemon balm, one of the important medicinal plant species mainly grown in natural flora -especially in Mediterranean region- of Turkey, is native to southern Europe and northern Africa, and east as far as the Caucasus and northern Iran [3]. It is a perennial plant growing up to 100 cm and 3 years old. The soft, hairy leaves are 2 to 8 cm long and either heart-shaped. The leaf surface is coarse and deeply veined, and the leaf edge is scalloped or toothed [1].

As a result, consumer's interest in natural products, especially plant extracts. Essential oils and/or their components are becoming increasingly popular as natural antimicrobial agents to be used for a wide variety of purposes, including food preservation, complementary medicine and natural therapeutics. Medicine comprises numerous herbal prescriptions for therapeutic purposes,

which may vary as healing, wounds, treating inflammation due to infection, skin lesions, leprosy, diarrhea, scabies, venereal diseases, snakebite etc [3].

Today, it is used in different branches of industry (such as medicine, perfume, cosmetic, and food etc.) it is currently used in medicine and pharmacology (anti-tumor, anti-bacterial, antimicrobial, antihistaminic, antispasmodic and antioxidant, by means of its antiviral effect curing of the herpes, anticancer, moderate Alzheimer's disease, modulation of mood and cognitive performance, stimulating the immune system (against anti HIV-1). In addition, lemon balm has traditionally been used due to its memory enhancing properties, but using of which currently more is widely used as sedative and antibacterial agent and sleep aid has been more popular recently.

In many countries of the world during latter years many assays that were acquired new antibiotic that thought medicine useful performance and this job was caused production more thousand antibiotic [4]. Resistance process bacteria than chemical antibiotic ability physicians at treatment some infection daises that most mortal has been confined [5]. Target of this in research has been investigated affects ethanol extraction and essential oil *Melissa officinalis* (Lemon balm) on *E. coli*.

Materials and Methods

That investigation of strain standard *E. coli* that from Institut scientific and industrial of iran with PTCC 2157. Culture medium was used muler hinton agar and nutrien broth (Merck; Germany). Antibiotic disk was used contain cifaxime, ceftriaxone, ciprofloxacin (Mast; England).

Dried leafs this plant was bought from research farmer Esfahan station. For isolation of the ethanol extraction, leafs plant were carefully cleaned in scrutiny laboratory university of falavarjan and then ethanol extraction used in the succilation- Rotary system. The weigh 10g of the powder and then specific paper with 100% and 80% ethanol was infused [7]. The isolation of the essential oil, Clevenger apparatus was used. The leaf's were carefully cleaned and distilled separately for 2 h. The main components of lemon balm essential oil, ranged from 0.01 to 0.25%, are 39% citronellal, 33% citral(citronello, linalool) and geraniol. The essential oil content in the leaves of respective herbs was in the range 0.39%- 0.14% V/m (top third part-whole aerial part) [7]. For saturation disk essential oil and ethanol extraction of nomination sensitivity test blank disk was used initial with DiMethyl Sulfooxide (DMSO) 99% various ethanol extraction concentrations 50, 100, 200, 400, 600, 800, 1000 mg/ml and essential oil concentrations 50, 25, 12.5, 6.25, 3.12% was prepared and sequence on the disks content 40 µl, 10 µl was inoculated. At scrutiny falavarjan laboratory of cultures 24 h bacteria were cultured in NB, 0.5 mac farland was prepared and with lop on the MHA plate was cultured, then was put disk on the plats. Results next for 24h was compared with cifaxime, ceftriaxone, ciprofloxacin, this work 4 was repeated.

Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) was used 96 whole plate ELIZA. Also, various concentrations of essential oil was added and then at one of the whole 50µl broth nutrient and then 10µl of the concentrations essential oil was affixed and then mixed and then 40 µl bacteria 0.5 mac farland was affixed and then for 24 h incubation at deep seated plates the grows of bacteria quite was detected. In the 5 whole was existed grows of bacteria and then previous concentrations cultures on the pelate one of the whole does not grows of bacteria was meet in rubric MBC that to 4 lodge was meet .then quantity was MIC 3×10^{-3} % and MBC 6×10^{-3} %. Comparison between group instance and care was used ANOVA. For study statistical assay results of SPSS 14 plan and graphs with Excel was used.

Results

Respondent in this investigation diameter against various ethanol extraction concentrations next for 24 hour in *E. coli* diameter of inhibitory zone was zero approximately, but average inhibitory zone against concentrations essential oil was 30mm approximately (Table 2, Fig. 1). Also in this investigation diameter of inhibitory zone against antibiotics cifaxime, ceftriaxone,

ciprofloxacin at was shown at table3 and the end of average inhibitory zone various concentrations essential oil in figure 2 was exhibited.

Table 1. Ethanolic extraction with antibacterial activity against *E. coli* (values in italics indicate DIZ values)

Ethanolic extraction (mg/mL)	50	100	200	400	500	1000	DMSO (100%)
Diameter of Inhibitory Zone(mm)	3	3	3	3	3	3	3

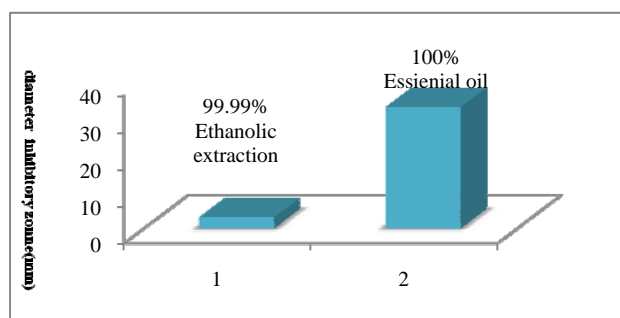


Figure 1. Comparison ethanolic extraction and essential oil *Melissa officinalis* L. grows of the *E. coli*

Table 2. The diameter of inhibitory zone *E. coli* against essential oil *Melissa officinalis* L. (mm)

Concentration of essential oil (%)	100	50	25	12.5	6.25	3.12	DMSO (100%)
DIZ(mm)	33	17.2	13.8	11	3	3	3

Table 3. The diameter of inhibitory zone *E. coli* against antibiotics Cifaxime, Ceftriaxone, Ciprofloxacin (mm)

Antibiotics	Content of disk (mcg)	DIZ(mm)
Cefixime (CFM)	5	22
Ceftriaxone (CRO)	30	28
Ciprofloxacin (CIP)	5	32

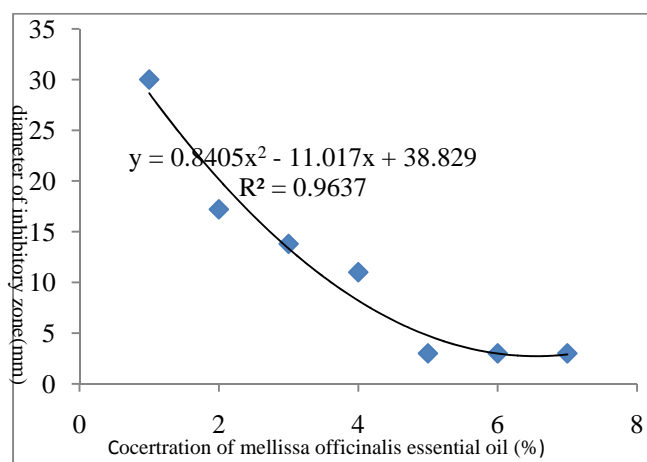


Figure 2. The diameter of inhibitory zone *E. coli* against different concentration essential oil *Melissa officinalis* L.

Table 3. Comparison diameter of inhibitory zone *E. coli* against different concentration essential oil *Melissa officinalis* L. With antibiotics

Concentration of essential oil (%)	DIZ	Standard deviation	Comparison with cefixime (20 mm)	Comparison with ceftriaxone (28 mm)	Comparison with Ciprofloxacin (32 mm)
12.5	11	1	0.001	0.001	0.001
25	13.8	1.6	0.001	0.001	0.001
50	17.2	2.3	0.052	0.001	0.001
100	33	1.8	0.001	0.01	0.98

Discussion

According to the statistical analysis gained from the data, there is a direct relationship between diameter of inhibition zone and concentrations essential oil with decrease concentration essential oil the diameter of inhibition zone was significantly decrease ($p=0.001$) and the diameter of inhibition zone was different all of the concentrations ($p=0.05$) (Fig.2). This process shows that the essential oil of this plant has a certain antibacterial effect, increasing with the increase in the effective substance. According to the statistical analysis, there is no significant difference at concentrations essential oil 12.5%, 25%, 50% with antibiotics cefixime, ceftriaxone, Ciprofloxacin ($p=0.001$). There is significant difference at concentration essential oil 100% at comparison with antibiotic cefixime ($p=0.001$) thereupon diameter of inhibition zone antibiotic cefixime most of this concentration essential oil. Also there is significant difference at the same concentration essential oil diameter of inhibition zone and antibiotic ceftriaxone and diameter of inhibition zone at antibiotic ceftriaxone most of the this concentration essential oil and inhibition zone at antibiotic and this concentration essential oil was shown (Table3).

According to the statistical analysis gained, quantity was MIC 3×10^{-3} % and MBC 6×10^{-3} %.

Nowadays with the side effects of antibiotic and safety of herbal combinations, attention has been drawn to medical herbs. At this investigation effect of essential oil and ethanol extraction *Melissa officinalis* and then was shown that essential oil more have effect of into some antibiotic on *E. coli* but diameter of inhibition zone into ethanol extraction was zero approximately. Considering the fact that studies of scientists indicated that *S.aureus* is one of the important factors in the infections urine [7, 8] the essential oil of this plant can be used to encounter this infection in the *In vivo* conditions. Since Ciprofloxacin is one of the antibiotics prescribed to treat this disease [9], one of the most common side effects of this include digestive and neural problems [10], this plant is so relaxing and quantity is MIC very low in the broth and must quantity will acquire at qualification *in vivo*.

Herodez and et al. with use HPLC was shown that combination ethanol extraction *Melissa officinalis* inclusive kaempferol methyl ether and three other combinations ursolic acid, Rosmarinic acid methyl ester, carnosic acid that strong antioxidant. Aromatic combinations such as citronellol, citronellal a low quantity was analyzed [9]. This scrutiny pay attention to at was used ethanol extracted. The essential oil has aromatic mixture and antibacterial effect.

Also one of the mechanism transmission signals in phagocyte is production free radicals, and then with assistance essential oil this plant may was not secrete any splutter of the phagocytes and too badly imposition that reason was destroyed tissues of human [10, 11].

Also studies of scientists was asserted *E. coli* one of the indicator sanitary, that pollution with *E. coli* was on sanitary at stages preparation and production [12, 13].we can will use from essential oil this plant for retentive of food.

De Sousa et al. performed the study on antitumor and antioxidant activities of lemon balm essential oil. The chemical composition and the biological activities of lemon balm essential oil obtained under controlled harvesting and drying conditions. Obtained findings showed that this oil was very effective against a series of human cancer cell lines (A549, MCF-7, Caco-2, HL-60, and K562) and mouse cell line (B16F10). Also, this result was shown that this essential oil contain activity antioxidant and mechanism that effect of on the free radical [14]. This result was shown that effect of mechanism thorough activity free radical and creation apoptosis. These results were shown that essential oil *Melissa officinalis* an agent antimicrobial.

Savino investigated that the colic in breastfed infant. The result of their studies shows that the colic in breastfed infant improves within 1 week of treatment with an extract based on *Matricariae recutita*, *Foeniculum vulgare* and *Melissa officinalis*. The essential oil of *Melissa officinalis* can uses of essential oil this plant for treatment this disease that some deal dependent at coliform at specific *E. coli* [15].

Studies of Mimica-Dukic et al. in 2004 have indicated that the antibacterial activity of essential oil against Gram-negative (eight strains) is shown. However, this study also recorded a notable susceptibility of examined Gram-negative pathogenic bacteria; such are *Pseudomonas aeruginosa*, *Escherichia coli*, *Salmonella enteritidis*, *Salmonella typhi*, and *Shigella* strains, particularly the multiresistance ones. Especially considerable is that the highest sensitivity to essential oil of *M. officinalis* was observed by *E. coli* ATCC 25922 (30.2 and 39.8 mm) and the multiresistant strain of *Shigella sonnei* IPH-MR (37.4 and 38.4 mm). (essential oil 20%, 50% concentration) Essential oil was diluted with n-hexane (solvent expressed no antimicrobial activity) [15]. In this investigations instance lineage for study was a little different lineage this study and concentrations essential oil was a little different this study, but quantity MIC and MBC to nutrient broth the different result was shown of this study also diameter of inhibitory zone was

zero approximately against various ethanol extraction concentrations.

Studies of Stanojevic scrutiny activity antibacterial ethanol extraction and Water extract *Melissa officinalis* was shown and synergic mechanism those with retentive of food for example sodium nitrat , sodium banzovat and potasium sorbat a against bacteria that author putrefy was studied of foods that could of this plant to industrial was used effect of mechanism synergic reaction those to any polution extracts plant and retentive of food was attend. Effect of synergic extraction asserted that MBC extract *Melissa officinalis* and retentives sequence was 1.4, 1.8 mg/ml [9, 16]. This result scrutiny and effects antimicrobial essential oil *Melissa officinalis* can uses of essential oil this plant instead of chemical retentive for retinue food. At the end of this finding may with was

performed more experience that qualification *In-vivo* of the essential oil *Melissa officinalis* will use in the pharmacy industry and food industry.

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Authors' Contributions

All authors had equal role in design, work, statistical analysis and manuscript writing.

Conflict of Interest

The authors declare no conflict of interest.

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